Portable Digital In-line Holography Platform for Sperm Cell Visualization and Quantification

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Abstract—In this paper a new portable Digital In-line Holography Platform for biological micro-scale imaging of sperm samples is presented. This platform is based on the shadow imaging principle, where biological samples are illuminated by a nearly coherent light source, and shadows are recorded into a CMOS imaging sensor with no lens requirement. The projected shadows present holographic signatures, storing more than bidimensional information of the analyzed sample. To improve resolution and suppress noise of the acquired holograms, a multi-frame technique based on high-dynamic range imaging combined with summation of consecutive frames over time was used. Finally, decoding of holograms is performed by an efficient and fast phase-recovery method, where morphological details of the sample can be obtained similarly a conventional brightfield microscopy image. From an image formation point of view, the proposed portable approach is able to visualize biological samples in high synthetic numerical aperture values with a spatial resolution of 1–2 μm on a field-of-view of \approx 30 mm^2 . This fieldof-view is consistently bigger than a conventional microscope imaged area with no mosaic reconstruction, and the achieved resolution is obtained using a single illumination source with no moving parts, array of LEDs or sift of the light source. Validation of the proposed portable approach was performed using various human samples, where conventional microscopy was used for confirmation purposes.

Keywords-Biological On-Chip Imaging; Digital In-line Holography Platform; Lens-less Shadow Imaging; Sperm Morphology Visualization.

I. INTRODUCTION

In the last years, with the recent achievements in nanoscale technologies, several miniaturized portable platforms for health-care applications have been proposed. For the homecare context, such platforms are very attractive solutions specially in the monitoring of specific chronic diseases [1]. In developing countries or resource-limited settings, portable platforms play a very important role where inexpensive, reliable and user-friendly diagnostic capabilities at the pointof-care (POC) can be used for the treatment and monitoring of a series of infections diseases [2], [3], [4]. Additionally, according to the World Health Organization (WHO), such platforms should be easy to use and robust operating even under adverse conditions [5], [6].

While conventional microscopy usually requires an advanced infrastructure and are mostly restricted to clinics and established hospitals, portable imaging devices can be used on general conditions within acceptable efficiency requirements. A significant progress in charge-coupled devices (CCD) and complementary metal-oxide-semiconductor (CMOS) sensors has been achieved, and in nowadays affordable imaging sensors with good resolution and field-of-view (FOV) are available. CMOS sensors are popularly used in the mobile industry and they can be easily miniaturized to operate in smart-phones or tablets. Also, CMOS are inexpensive when compared to the available CCD technology, and they can operate with reduced energy requirements.

Portable imaging devices for health-care applications and biological imaging can be also observed in the literature. A very simple portable approach for biological imaging can be achieved by using the imaging sensor of a conventional smart-phone integrated with some optical settings [7], [8]. Algorithms for image interpretation can be further designed for automatic counting and differentiation of findings for the diagnostic process. Advantage of this category of portable imaging devices is the wide availability of the recently developed smart-phones, but the FOV is very limited due the embedded optical settings. A very recent discussion and trends for biological imaging using smart-phones is presented in [9].

Another category of biological imaging in a wide FOV is based on the shadow imaging principle, enabling the investigation of samples directly over the imaging sensor surface with no lenses requirement [10], [11], [12], [13]. This is done by using conventional CCD or CMOS based imaging sensors, where the sample to be analyzed is positioned directly on the top of the sensor surface. A light source positioned above is used to illuminate the sample, projecting shadows of the intercepted targets on sensor cells where image is formed. This technique was initially proposed by [10], where a ultrawide lens-less imaging platform using a CCD camera for



automatic cell counting and discrimination was used. The great advantage of this category of imaging platform is the wide FOV with no lens requirement (usually the area of the imaging sensor), besides the need of specific setup composed by imaging sensor, light source and a computational resource for image analysis.

Based on the previously mentioned shadow imaging principle with small physical modifications, spatial resolution can be greatly improved by using in-line holography principle proposed by Gabor [14]. This principle, similar to the early stages of human vision for texture discrimination, is achieved by using the wave-diffraction theory, where a reduced wave-length light-source is used to achieve a nearly coherent illumination system. When waves intercept targets, holographic signatures are projected corresponding to the encoded morphological details of the analyzed sample. The obtained holographic signal has more than bi-dimensional information (i.e., morphological information from different heights can be reproduced), and computational phase-retrieval methods can be further applied to decode the holographic signals into morphological information, recovering shape and topological details of the intercepted target. This category of biological holographic imaging is known as Digital In-line Holography Microscopy (DIHM), and it differs from the traditional holographic principle, where the mother wave should be separated from the sample beam [15].

In this paper we present the results of a new portable lens-less wide-field DIHM approach able to capture and solve challenging non-circular holographic signals produced by sperm samples. Sperm morphology is $3\mu m$ and $5\mu m$ of head, and tail corresponding to $50\mu m$ by less than $1\mu m$ of thickness, presenting unique features allowing to be easily identified in our approach even without microscopy confirmation. Differently from the state-of-the-art computational approaches, holograms are captured by using only a single coherent illumination source and obtained holograms are improved by a combined High-Dynamic Range (HDR) image approach with noise suppression provided by summation of static observations over time. To circumvent computational limitations in execution time of the commonly used iteractive phase-retrieval methods, we are using a fast Angular Spectrum Method, providing holographic decoding at different height positions. The proposed computational approach was evaluated considering very small sperm human cells whose dimensions are in the order of a few microns. Obtained results show a spatial resolution of $1-2\mu m$ on a field of view of $\approx 30mm^2$. Validation was performed using bright-field microscopy images for confirmation purposes. Effectiveness of the proposed approach can be observed by the visualization of very small structures such as sperm tails, a very challenging task when performed by a lensless portable platform. This is the first portable lensless imaging platform for sperm morphology visualization using a single and fixed light-source to produce holograms, where no array of LEDs or sift of the light-source for pixel super-resolution was used.

II. RELATED WORKS

Recently in the literature several works describe the use of DIHM for the investigation of biological samples. A large number of approaches are dedicated for counting or cell discrimination, whose dimensions are in the order of 10 μm [11], [16], [12], [15]. Using a different approach, a lensfree holographic imaging platform to perform on-chip cytometry is presented in [11]. In this work, recorded holographic image is processed by using a custom developed decision algorithm for matching the detected hologram texture to existing library images for on-chip characterization and counting of a heterogeneous solution of interest. There are approaches where super-resolved or pixel super-resolution is used to estimated a higher-resolution and to decrease pixel-size computationally. For these approaches, some kind of disturbance should be introduced over time to slightly chance the content of the captured holographic signal. In [17] a constant pressure scheme was used to displace the sample while imaged by a very high frame-rate camera device (1000 fps). Super-resolution methods were used to reconstruct a high-resolution image over time.

In [16], [18], an array of 23 LEDs and a micro-controlled device were used to sift the light-source and displace holographic signatures over the sensor surface. A higher resolution holographic image is obtained from 23 low-resolution by the minimization of an objective cost-function designed for image registration and deconvolution. Image interpretation and decoding is performed by an iteractive phase-retrieval method, allowing micro-scale visualization after a specified number of iteractions is achieved. Different human samples were analyzed in this approach presenting a FOV of $\approx 24mm^2$. However, this approach lacks on implementation details in how to effectively minimize the objective cost-function to achieve a high-resolution image.

Considering the visualization of non-circular biological shapes in a lensless portable platform, such as sperm samples, (scope of our work), only [16], [18] present effective results for sperm sample visualization using a lensless holographic platform, regarding the identification of morphological details such as head and tail. Later, using the same holographic platform, Su et.al. in [19], [20] demonstrate a novel high-throughput lensfree 3D tracking of human sperms using Lab-View software, based-on the immersion of live samples in a medium. Holographic video is recorded and further decoding is performed to track sperm trajectories over time. In this last approach, only sperm heads can be visualized once they are in movement.

When compared to the state-of-the-art methods, our approach has as contribution the visualization of morphological details by using a single and fixed illumination source, where no sift of array of LEDs is used to capture holograms. From a computational side, we are performing noise suppression with some resolution improvement using different exposure ranges available in the CMOS imaging sensor, where a HDR image is obtained. Diffraction is also performed by a non-interactive and fast Angular Spectrum Method, where the entire FOV of



Fig. 1. General overview of the proposed approach. (1) Schematic representation of the experimental setup developed to capture holographic images; (2) Multiple exposure range frames are acquired; (3) A HDR holographic image is obtained; (4) Angular Spectrum Method is used to decode holographic signatures; (5) Bright-field microscopy image (10x) is used for confirmation.

 $\approx 30mm^2$ is processed in a few seconds.

III. PROPOSED APPROACH

This Section presents the proposed DIHM approach considering hardware components and computational image processing methods. Figure 1 illustrates a general overview of the proposed approach. The image acquisition module regarding our experimental setup is shown in Figure 1-(1) and explained in the next sub-section. Here, as the imaging sensor has a proper SDK to control hardware instructions, a set of consecutive frames with different exposure values is obtained according to Figure 1-(2). To improve resolution and reduce noise, a HDR image approach is used to equalize brightness and contrast levels of holograms represented by periodic waves, according to Figure 1-(3). The objective of this step is to suppress noise and increase propagation of holographic signal, or in other words, the number of fringes corresponding to highfrequency signals. High-quality propagation allows to obtain better morphological details when decoded by phase-retrieval methods. This is done by the Angular Spectrum Method demonstrated in Figure 1-(4), where holograms are converted into morphological structures. Finally, but not necessarily a required step, a bright-field microscopy image is obtained from the same sample for confirmation and validation purposes, as illustrated in Figure 1-(5).

A. Experimental Setup and Biological Sample

In our approach the experimental setup developed to capture holograms is mainly composed by 3 parts as illustrated in Figure 1-(1), being an illumination source (LED), the sample to be analyzed inside of cover glasses, and an imaging sensor to capture holographic signals.

Illumination is performed by a LED of 315 mn of wavelength, model M385L2 from ThorLabs, positioned approximately 8 cm above the sensor and the sample. This light-source is a nearly coherent illumination and no pinhole is required when using this short wavelength, although a small numerical aperture can be used to reduce the distance between the lightsource and the sample / imaging sensor.

Several biological samples were used to validate the proposed approach, more specifically sperm samples placed between glass slides and positioned directly on the sensor surface. Sperm are male reproductive cells, with dimensions at the order of $3\mu m$ and $5\mu m$ of head, and tail corresponding to $50\mu m$ long, and they are particularly interesting presenting unique characteristics and morphology (head and tail), allowing to be easily identified even with no microscopy confirmation. For the analysis, $10\mu l$ of sperm solution was added on a glass slide, and mounted with a 8x8mm cover slip.

Imaging sensor is Aptina CMOS of 10 megapixel with dimensions corresponding to $6.4mm \times 4.6mm$, resolution of 3840x2748 pixels and 2.5 frames per second. Pixel size presented in the imaging sensor is 1.67 μm , and this sensor is connected by USB interface with proper SDK for image acquisition, where sensor instructions can be accessed, such as shutter time used for HDR imaging. In this imaging sensor anti-aliasing and pixel post-processing should be disabled. Also, there is no hardware support for HDR in this imaging sensor, and shutter time (exposure value) can be manually

adjusted to compute a better distribution of light intensities for holographic purposes. A homemade setup was built on a CO_2 laser cutter engraver to support the light-source and the imaging sensor. Specific chambers were built to ensure the direct contact of the analyzed sample and the CMOS sensor surface.

B. Image Acquisition and High-Dynamic Range Image

The image acquisition is performed by iterating different exposures values and storing its corresponding frames. As noise is introduced on each single frame and at the same time an image can be consistently degraded by noise, more details can be recovered by simply average of many exposure frames.

A common approach used to create a HDR image is by merging different exposures of the same scene, and thus bring images with low-dynamic range into the same domain. This is done by a normalization procedure where each image is divided by its corresponding exposure times. In a practical context, this process cannot be directly applied once most imaging sensors apply some kind of non-linear (post)processing to the incident light as it passes through camera circuitry [21]. Therefore, this non-linear process ideally must be known in advance and thus inverted before producing a HDR image. Usually this kind of response is proprietary and not disclosed by a camera manufacturer.

On the other hand, this non-linearity can be estimated by approaches specifically designed to compute an approximate response function based on a set of image sequences with different exposure values, such as [22], [23], [24]. This estimated function can be used to linearize the frames and a mean irradiance map can be obtained. During this process, pixels with no usefull information (under or over-exposed) can be eliminated from summation. Also, due to the non-linear processing of the imaging sensor, not all the pixels are equally reliable during the summation, so a weight function must be applied for each frame. Several weight functions were proposed in the literature to solve this problem and alter the weighting function to find useful pixels corresponding to a good compromise between noisy, under and over-exposed pixels. In our approach we are using a weight function proposed by [25], where the luminance of the pixels is used as the input to the broad hat function. This weight function is given by:

$$w(x,L) = f^{-1}(x)f'(x)\left[1 - \left(\frac{L}{127.5} - 1\right)^{1/2}\right], \quad (1)$$

where f(x) is the estimated camera response and L the luminance value. Based on the obtained weight function w, the irradiance value I_p of pixel p is computed as a weighted average of the corresponding pixel values in its N constituent frames by means of:

$$I_p = \sum_{a=1}^{N} \frac{f^{-1}(p_a)w(p_a)}{t_a} / \sum_{a=1}^{N} w(p_a).$$
(2)

According to the previous equation, p_a is the value of the pixel p in a frame a. The exposure value of the shutter time of a is then given by t_a .

In Figure 2 a comparison of the HDR approach for holographic signal improvement is presented. First and second columns show a single frame hologram and its HDR version, respectively. HDR image was produced using 10 consecutive frames with exposure times varying from 1/25 to 1/250 and step size of 1/25. For visualization purposes, a gradient image given by the Prewitt compass is demonstrated, where third and fourth columns correspond to the gradient of single and HDR holograms, respectively. At the bottom side, a zoomed area presents the main differences for noise suppression and recovery of high-frequency signal.

C. Angular Spectrum Method for Holographic Decoding

Diffraction calculation is the process of finding numerical computational solutions to the phase problem produced by the apparent effect of bending light waves when it intercepts obstacles and openings. Diffraction is the most important aspect of the holography, being practically used in wide-ranging optics fields, revealing important properties of diffracting objects, such as its geometry and shape.

In Fourier optics, diffraction calculations can be categorized by: (i) convolution-based diffraction and (ii) Fourier transformbased diffraction. One example of convolution-based for computing diffraction at short distances (i.e., plane and the imaging sensor) is the Angular Spectrum Method (ASM). ASM is a technique for modeling the propagation of a wave field by expanding a complex wave field into a summation of infinite number of plane waves. Given a recorded hologram signal whose physical properties are known in advance (such as distance and wave-length), the ASM can be expressed by [26]:

$$u(x,y) = \int \int_{-\infty}^{+\infty} A(f_x, f_y, 0) H(f_x, f_y)$$

$$\exp(i2\pi (f_x x + f_y y)) df_x df_y,$$
(3)

where f_x and f_y are spatial frequencies, $A(f_x, f_y, 0)$ is a Fourier transform $F[u(x_1, y_1)]$, and H is a impulse response function (transfer function). The discrete part of Equation 3 can be expressed by the following Fast Fourier Transform (FFT) and its inverse:

$$u(m,n) = FFT^{-1}[FFT[u(m,n)]H(m_1,n_1)].$$
 (4)

The discretizing f_x and f_y frequencies are respectively $(f_x, f_y) = (m_1 \Delta f_x, n_1 \Delta f_y)$, and m_1, n_1 are integer indices on the destination source. Δf_x and Δf_y are the detector sampling pitches on the frequency domain, i.e., the distance between the centers of adjacent detector elements.

The distance between the source plane (aperture function) $u(x_1, y_1)$ and the destination plane (sensor) is determined by z, used as a parameter of the algorithm equivalent to the focus at different height positions. The impulse response function H is given by:

$$H(f_x, f_y) = \exp(iz\sqrt{k^2 - 4\pi^2(f_x^2 + f_y^2)}),$$
 (5)

where $k = 2\pi/\lambda$ is the wave-number, and λ is the wavelength of the light-source. By varying z, diffraction responses at



Fig. 2. Single frame image compared against an improved HDR image. Prewitt compass applied to single and improved HDR image, respectively (right side), demonstrating better wave propagation and holographic improvement of fringes.

different heights equivalent to the focus are given. Also, real (\Re) and imaginary (\Im) parts can be obtained, and phase and amplitude can be computed by $\theta(x,y) = \tan^{-1} \frac{\Im(x,y)}{\Re(x,y)}$ and $r(x,y) = [\Re(x,y)^2 + \Im(x,y)^2]^{1/2}$, respectively.

Diffraction calculation at different heights is demonstrated by Figure 3, where a sperm hologram image was decoded by the ASM into \Re and \Im parts. Parameters for diffraction distance z vary from 30k to 38k related to the distance between the sample and sensor cells.

Additionally, in our approach we are able to determine z automatically by means of a focus measure responsible to compute the relative focus degree of an image. This is done by a simple sum-modified-Laplacian (LAPM) algorithm [27], which is based on two partial derivatives to measure the sharpness level of an image. Sharpness is arguably the most important photographic image quality factor because it determines the amount of detail an imaging system can reproduce.

An extensive validation of 33 focus measure methods have shown that Laplacian-based operators have the best overall performance at normal imaging conditions [28]. However, it is difficult to determine which focus measure operators have the best performance for specific imaging conditions, since this strongly depends on the particular capturing device with which the image sequences are acquired.

IV. EXPERIMENTS AND RESULTS

The proposed approach was mainly validated based on two aspects: (i) holographic signal improvement with noise suppression and (ii) bright-field microscopy image confirmation.

Holographic improvements can be verified in Figure 2, where the improved HDR image is compared against a single frame. Wave propagation or fringes play a fundamental role in diffraction calculation, increasing structural details for the recovered image. High-frequency waves correspond to boundary information of the intercepted targets, while low-frequency stores information related to the center of the intercepted objects. Noise suppression in holograms is equally important to remove undesirable artifacts included during the acquisition process. Noise is introduced by physical and environmental conditions, such as the oscillatory light intensities captured



Fig. 3. Diffraction calculation using the Angular Spectrum Method for sperm sample holograms. Focus measure is used to determine the diffraction result where sharpness level is optimized. Real (\Re) and Imaginary (\Im) parts are demonstrated by recovering the signal at different heights ($z^*\lambda$).

by the sensor cells. As the nature of noise is non-periodic and non-homogeneous over time, it can be suppressed by summation of consecutive frames. The both improvement effects can be observed on gradient counterparts of Figure 2.

The second and more important validation aspect is the microscopy analysis which is related to the image formation problem. For this purpose microscopy analysis was performed to demonstrated the effectiveness of the proposed approach when compared to a bright-field microscopy image, used as a ground-truth. As the scanned area provided by a conventional microscope is smaller than the FOV of the proposed approach, mosaic images were obtained using an inverted microscope Carl Zeiss Axio Observer-Z1 with 10x of magnification. Several sperm samples were imaged using the proposed approach with their corresponding microscopy confirmation. Figure 4 demonstrates a wide-field holographic image decoded by the ASM, where several sperm cells can be observed on the background. As the imaging sensor has dimensions of 6.4mm x 4.6mm, the FOV is $\approx 30mm^2$. In this area, some regions were selected for microscopy confirmation, as illustrated on the right side of Figure 4 (Proposed Approach and Microscopy Image). The obtained results show a very high correlation of the proposed approach when compared against its corresponding bright-field microscopy images.

Additionally, a resolution analysis was also performed by comparing the proposed approach using a synthetic U.S. Air Force resolution chart (USAF-1951), commonly used for calibration and to measure the degradation of a lens system. USAF-1951 was imaged by the proposed approach according to Figure 5, where holographic signal was projected on the sensor surface. Figure 5-(1) refers to the wide-field holographic image obtained on the entire sensor area, and (2) is a holographic area corresponding to the elements 6–



Fig. 5. USAF-1951 Resolution Chart. (1) Wide-field HDR holographic image. (2) Holographic elements 6–9 area is shown. (3) recovered signal using the ASM from (3) is illustrated. (4) Elements 8–9 corresponding to a scale of a few microns is shown for the HDR approach. (5) The equivalent zoomed area for a single frame with no HDR approach.

9. This area was applied to the ASM method for decoding, as illustrated in (3). Another internal area, corresponding to the elements 8–9 was selected, as shown in (4), and in this area the charts refer to micro-scale presenting a visible



Fig. 4. Wide-field holographic image of $\approx 30mm^2$. At left side a hologram and recovered signal part is shown. At the right side, recovered signal from different parts in the wide-field image is demonstrated, validated with bright-field their corresponding microscopy image.

resolution of less than 1.54 μm (element 8, section 3, with 323 line pairs per mm). Similarly, a comparison using a single frame was performed for comparison with no HDR and not applied to noise suppression improvements, as demonstrated in Figure 5-(5). Although the obtained resolution results were within acceptable ranges, USAF-1951 chart is traditionally used for imaging systems based on lens, but not optimized for holographic signals. We can observed this fact when Figure 5-(2) is analyzed: holographic signals from neighboring charts are projected on each other, so very small elements may have their information reduced. This kind of effect was observed

when a very high dense experiment with red blood cells was performed, confirming the fact that holographic information may be limited by the density (dilution) of the analyzed sample. On the other hand, when density can be controlled, as demonstrated in Figure 4, at the left side (holograms and recovered signal), interference is minimized and the Angular Spectrum Method can provide very interesting results in terms of resolution.

V. CONCLUSION

In this paper we demonstrated a new portable lens-less wide-field microscopy platform for sperm sample visualization based on multi-frame HDR image combined with Digital Inline Holography.

Portable imaging devices are very attractive solutions for Point-of-Care applications, with inexpensive cost when compared to a conventional microscope. In this paper, a CMOS imaging sensor was combined with computational HDR imaging and phase-recovery methods for biological imaging investigation using single shots with different exposure ranges, a single light-source and no moving parts. In the literature only one related work was found using lensless holography for effective visualization of sperm morphology on a FOV of $\approx 24mm^2$ [16], [18], but using super-resolution and a microcontrolled array of LEDs to sift the light source.

In our approach, the obtained results show a spatial resolution of 1–2 μm on a FOV of $\approx 30 mm^2$, and validation was conducted using corresponding bright-field microscopy images, showing a very high correlation of sperm samples, which are very small human cells in the order of a few microns.

CMOS sensors are preferable for POC applications due its reduced dimension and no requirements for external energy supply (can operate via USB connection). Another positive aspect is a CMOS technology can avoid excessive warming on sensor surface where samples are places, when compared to CCD technology (usually a cooling system is required). Lensless imaging technologies are best suited for the development of portable POC solutions when combined with high-level pattern recognition techniques, making the proposed approach practical for low-cost real-world applications.

Further studies should consider the development of methods for automatic identification and classification of sperm samples, as well as other kind of biological samples. Also, twinelimination problem should be considered to remove holographic artifacts observed after diffraction method is applied.

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